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J Anim Sci 2004. 82:3380-3387.

Testosterone and luteinizing hormone responses to naloxone help predict sexual performance in rams[1]

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ABSTRACT: The first objective of this study was to determine whether LH and testosterone respond differently to a naloxone injection in relation to varying sexual performance in rams. If differences occurred, the second objective was to determine whether differences would predict variation in sexual performance. From a group of 1.5- to 3-yr-old rams, 20 sexually active and 39 sexually inactive rams were selected based on previously observed sexual behavior with estrual ewes. Each ram was exposed to three estrual ewes for 18 30-min sexual performance tests, and those found to be inactive were given two 30-min sexual partner preference tests. The final distribution was 28 sexually active, 22 inactive, and nine male-oriented rams. Rams were treated with 1.5 mg of naloxone/kg BW in December of Year 1 and again with either 0.75 or 1.5 mg of naloxone/kg BW in November of Year 2. Plasma concentrations of LH and testosterone were evaluated with mixed model analyses for repeated measures separately for each year to coincide with logistic procedures for modeling the probability that rams were sexually active. For Year 1, a sexual activity × age × time interaction for LH after naloxone was observed ($P < 0.03$). For testosterone, there was a sexual activity × time interaction ($P < 0.03$), with a similar, early increase for sexually active female- and male-oriented rams compared with a delayed, minimal increase for inactive rams. For Year 2, when all rams were over 2.5 yr of age, a sexual activity × time interaction for both LH and testosterone ($P < 0.02$) seemed more related to an earlier increase of both hormones for sexually active rams than the increase observed for inactive rams. In addition, sexually active rams had a greater increase in testosterone than inactive rams. No significant difference was observed between 0.75 and 1.50 mg of naloxone/kg BW. Testosterone and LH were used as explanatory variables and sexual activity was used as the response variable in logistic procedures. In Year 1, greatest prediction accuracy was 73.5% using testosterone at 60 min after naloxone injection. In Year 2, the greatest prediction accuracy was 85% using LH at 15 min multiplied by testosterone at 60 min after naloxone. Test repeatability for both years on the same rams was 76%. In conclusion, pattern and magnitude of naloxone-induced changes in endocrine function may facilitate identification of sexually active and inactive rams during the breeding season. Prediction accuracy of the naloxone-based test was 69 to 85%.

Key Words: Luteinizing Hormone, Naloxone, Rams, Sexual Performance, Sheep, Testosterone

 J. Anim. Sci. 2004. 82:3380–3387

Introduction

Sexual performance varies among rams (Terrill, 1937; Hulet et al., 1962; Price, 1987). Variability of sexual performance ranges from rams with no interest in mating to those exhibiting a high capacity to mate repeatedly with many ewes. The serving capacity test (**SCT**) developed by Kilgour and Whale (1980) has been used extensively to evaluate sexual performance in rams and involves exposing rams individually to estrual ewes for a specified time. Rams can be ranked from low to high sexual performance by counting the number of ejaculations achieved during the period. A series of SCT is a good predictor of breeding performance (Perkins et al., 1992b), but SCT are labor-intensive, expensive, and often impractical to conduct. Clearly, an easier and more practical method would be advantageous.

Naloxone (an opioid inhibitor) induced copulatory behavior when injected into sexually inactive rats (Gessa et al., 1979) but did not result in increased sexual activity when injected into sexually inactive rams (Fitzgerald and Perkins, 1994). However, it was noted that there seemed to be differences in LH response to naloxone in sexually active and inactive rams. It was postulated that testosterone would also respond differen-

[1]We cordially thank W. J. Gardner and M. A. Williams for their excellent technical assistance and B. E. Mackey for statistical advice.

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Received January 23, 2004.
Accepted August 5, 2004.

tially and that these hormone response differences could be the basis for a test to identify sexually inactive rams. Therefore, the objectives of this experiment were to 1) determine whether LH and testosterone respond differently to naloxone depending on the sexual activity status of rams; and 2) if differences occurred, to determine whether they could be used to predict sexual performance of the rams.

Materials and Methods

General

All rams and ewes were kept at the U.S. Sheep Experiment Station, Dubois, ID (long 44° 14′N, lat 112° 11′W). Rams were raised in an all-male setting after they were weaned at approximately 120 d of age as a typical management practice. Rams and ewes were offered long-stem alfalfa hay (relative feed value of 165) at 2.2% of BW (DM basis) daily, housed as a group in an outside paddock, and given free access to water and trace mineral salt (Redmond T.M. [2,000 ppm Mn, 3,500 ppm Zn, 600 ppm Fe, 300 ppm Cu, 80 ppm I, and 50 ppm Co]; Redmond Minerals, Inc., Redmond, UT) from September to April each year. For the remainder of the year, rams and ewes were maintained in independent groups according to sex of animal on native sagebrush grasslands and given free access to water and trace mineral salt (Redmond NTM [5 ppm Mn, 3 ppm Zn, 300 ppm Fe, 3 ppm Cu, and 10 ppm I]; Redmond Minerals, Inc.). The ewes were ovariectomized and estrus-induced using 60 mg of 6α-methyl-17α-hydroxyprogesteroneacetate pessaries (Pharmicia and Upjohn, Kalamazoo, MI) and estrogen (0.5 mL of 100 μg estradiol 17β/mL) as explained in detail previously (Stellflug and Berardinelli, 2002). Ewes were considered to be in estrus when they would stand to be mounted by a ram.

Sexual Performance Tests

The same rams were treated with naloxone each of two consecutive years in December and November, respectively. Before the naloxone treatments, 59 rams were selected based on three types of sexual performance tests: 1) a preliminary screening test in which rams were observed for 30 min with three estrus-induced ewes (Snowder et al., 2002); 2) a series of nine 30-min SCT (Stellflug and Berardinelli, 2002), in which rams were observed with three estrus-induced ewes; 3) a 30-min sexual preference test (Stellflug and Berardinelli, 2002), in which only sexually inactive rams were observed with two restrained estrual ewes and two restrained rams. Rams were classified as sexually inactive (no ejaculations) or active (one or more ejaculations). Rams given preference tests were classified as sexually active with ewes (female oriented), exclusively sexually active with rams (male-oriented), or as sexually inactive.

Thirteen 2.5- to 3-yr-old rams (3-yr-old rams) and 46 1.5-yr-old rams were assigned according to sexual behavior characteristics for the first year of the study. The 3-yr-old rams had had nine SCT, and those found sexually inactive were further evaluated with one sexual preference test. The 1.5-yr-old rams had only one screening test at initial assignment, and sexually active and inactive rams were equally represented. Sexual orientation of 1.5-yr-old rams was unknown at the time of assignment.

After the second year of naloxone treatment, all rams were tested again until all rams had a total of 18 SCT and all sexually inactive and male-oriented rams had a total of two preference tests. This resulted in a final breakdown of nine Columbia (one sexually active, eight sexually inactive), 10 Targhee (seven sexually active; two sexually inactive; one male-oriented), 24 Polypay (15 sexually active, four sexually inactive, five male-oriented), and 16 Rambouillet (five sexually active, eight sexually inactive, three male-oriented). Sexually active, female-oriented rams averaged from 0.5 to 3.8 ejaculations over the 18 SCT. One sexually active ram and two sexually inactive rams were eliminated for health reasons the second year. One ram was excluded from the data set for evaluating testosterone response in the first year because of missing testosterone data, but was included in LH analysis for the first year and for LH and testosterone for the second year.

Naloxone Administration and Blood Collection

The same rams were treated with 1.5 mg of naloxone/kg BW in December the first year and with either 0.75 or 1.5 mg of naloxone/kg of BW in November the second year. The initial dose of naloxone was based on the amounts given previously to rams (Schanbacher, 1985; Lincoln et al., 1987) that resulted in an LH response. In the second year, half the rams selected at random received 0.75 mg of naloxone/kg BW and the other rams were given 1.5 mg naloxone/kg of BW to determine whether the smaller dose was as effective at stimulating an LH and testosterone response as the greater dose.

Four blood samples were taken via indwelling jugular catheters (16 gauge, 1.7 mm × 133 mm; Angiocath, Becton Dickinson, Sandy, UT) at 15-min intervals before treatment. Naloxone was administered through the catheter immediately after the fourth blood sample. Four blood samples were collected at 15-min intervals after the naloxone treatment. The blood was drawn with 6-mL syringes and transferred into 5-mL glass tubes containing two drops of heparin (50 USP units of H3393 porcine heparin/mL physiological saline; Sigma, St. Louis, MO). Plasma was harvested and stored at –20°C until assayed for LH and testosterone concentrations.

Hormone Assays

Plasma LH concentration was quantified using a validated RIA procedure (Perkins et al., 1992a) that in-

cluded antioLH AFP-192279 and oLH AFP-8614B for iodination and standards, which were obtained through National Hormone Pituitary Program, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. The intra- and interassay CV were less than 10.3 and 4.0%, respectively, for the assays for the 2 yr, with a sensitivity of 0.25 ng/mL.

Plasma concentrations of testosterone were quantified with a commercial assay kit provided by ICN Biomedicals, Inc. (Costa Mesa, CA) and with plasma from castrated rams to dilute testosterone standards as previously validated (Perkins and Fitzgerald, 1992). Cross-reactivity was 3.4% with 5α-dihydrostestosterone. The intra- and interassay CV were less than 4.2 and 4.0%, respectively, for the assays for the 2 yr, with a sensitivity of 0.2 ng/mL.

Statistical Analyses

Plasma LH and testosterone concentrations were analyzed separately for each year using mixed model procedures of SAS (SAS Inst., Inc., Cary, NC) for repeated measures. The separate yearly analyses coincided with separate SAS Proc Logistic analyses for each year because logistic procedures process rams for both years as individual observations. The LH and testosterone data were evaluated for four samples before injection of naloxone, and for four samples after naloxone treatment in addition to the sample collected immediately before the naloxone treatment in separate analyses for each hormone. The corresponding average values before naloxone were used as a covariate in the analyses. The main plot for the first year included sexual activity (sexually active, sexually inactive, and male-oriented rams) and age of the ram. The subplot included time, sexual activity × time, age × time, and sexual activity × age × time. Sexual activity and age were tested with rams nested within sexual activity and age. In the second year, all ages were combined because all the rams were 2.5 yr of age or older. The main plot for the second year included sexual activity (sexually active and inactive rams) and dose of naloxone. The subplot included time, dose × time, sexual activity × time, and dose × sexual activity by time. Sexual activity and dose were tested with rams nested within sexual activity and dose. The subplot was tested with the residual. Degrees of freedom were calculated using the Kenward-Roger procedure (Kenward and Roger, 1997). The LH and testosterone values were transformed to the natural logarithm to normalize the variances among rams for the fixed effects. Least squares means and confidence intervals for LH and testosterone were changed back to original units after analysis. A standard error for the original units was estimated using the 95% confidence intervals; this was an approximation (approximate SEM) and not appropriate for estimating confidence intervals for means that would asymmetrically match the data distribution. If the main effects or their interactions were significant ($P < 0.05$), Fisher's protected least significant difference was used as a postanalysis test to detect differences between individual means.

Initially, to predict sexual activity of rams after a naloxone injection, the LH and testosterone data were evaluated subjectively by observing the hormone data and sexual activity to select an index referred to as the "naloxone index," where the highest prediction accuracy was achieved. The naloxone index consisted of LH concentration at 15 min after naloxone treatment multiplied by the testosterone concentration at 60 min after injection. Rams were categorized as sexually active if the naloxone index was equal to or greater than 5.4 and sexually inactive if the index was less than 5.4. This was the method used for the patent titled "Method of Sire Selection Using Naloxone Challenge Test and Kits Thereof" (Perkins et al., 2001).

Subsequently, sexual activity was used as a response variable, and LH and testosterone concentrations were used as explanatory variables using a more objective logistic procedure (Johnson, 1998; Stokes et al., 2000) for modeling the probability that rams were sexually active. Hormone responses to naloxone between sexually active and inactive rams from each year were evaluated separately. The hormone responses for the male-oriented rams were not included in the data set because the logistic procedure can accommodate only two levels for the response variable. The average hormone concentration for each time period was evaluated in addition to the combination of LH and testosterone based on the best subjective value related to the patent. The logistic regression model was ram class = LH sample time (**LHST**) 1 to LHST8 and testosterone sample time 1 (**TST1**) to TST8/selection = forward slentry (significance level for entry of variables into the model) = 0.10, hierarchy = single. The LH and testosterone profiles are presented in figures for general observation of the trends; however, primary evaluation of this test was based on the statistical analyses and logistic procedure.

Results

First Year

The significant covariate for LH before naloxone injection ($P < 0.001$) indicated an improved precision of the test for fixed effects after naloxone. The sexual activity × age × time interaction for LH after naloxone injection ($P < 0.03$) seemed to be primarily related to varied differences over time and secondarily related to sexual activity (Figure 1).

The covariate for testosterone before naloxone injection was significant ($P < 0.001$). A sexual activity × time interaction was observed after naloxone treatment ($P < 0.003$). The interaction seemed to be related to a dramatic increase ($P < 0.01$) in testosterone concentration beginning 30 min after naloxone in sexually active female- and male-oriented rams that remained elevated out to 60 min (8.0 ± 1.3 and 8.2 ± 1.8 ng/mL, respectively) compared with the delayed increase in sexually inactive

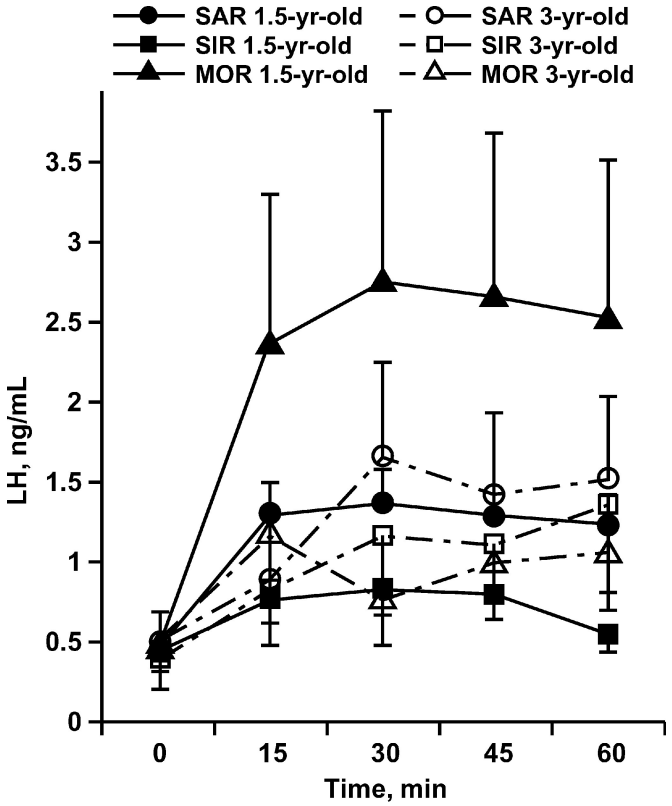

Figure 1. Least squares means for LH after treatment (1.50 mg of naloxone/kg of BW) during Year 1 for 1.5-yr-old sexually active rams (n = 23; SAR 1.5-yr-old [-●-]), 3-yr-old sexually active rams (n = 5; SAR 3-yr-old [-○-], 1.5-yr-old sexually inactive (n = 19; SIR 1.5-yr-old [-■-]), 3-yr-old sexually inactive rams (n = 3; SIR 3-yr-old [-□-], 1.5-yr-old male-oriented rams (n = 4; MOR 1.5-yr-old [-▲-]), and 3-yr-old male-oriented rams (n = 5; MOR 3-yr-old [-△-]. The sexual activity × age × time interaction was observed ($P < 0.03$) after naloxone; only those data are depicted in the figure. The estimate of variability is depicted by estimated standard errors of the least squares means because of data transformation.

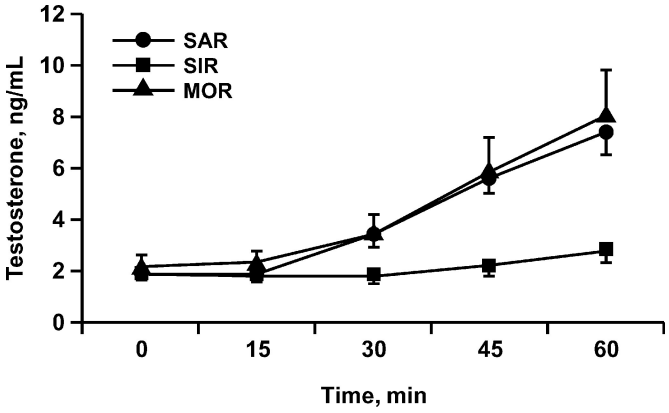

Figure 2. Least squares means for testosterone after treatment (1.50 mg of naloxone/kg of BW) during Year 1 for sexually active (n = 27; SAR [-●-]), sexually inactive (n = 22; SIR [-■-]), and male-oriented rams (n = 9; MOR [-▲-]). A sexual activity × time interaction was observed ($P < 0.003$), with SAR and MOR differing ($P < 0.01$) from SIR, but not from each other; only those data are depicted in the figure. The estimate of variability is depicted by estimated standard errors of the least squares means because of data transformation.

rams beginning at 45 min and the minimal increase at 60 min (3.3 ± 0.6 ng/mL) after naloxone treatment (Figure 2). Testosterone concentrations did not differ between female- and male-oriented rams.

For the older rams (2.5 and 3 yr olds), LH only varied over time, increasing from 0.5 ± 0.1 to 1.3 ± 0.3 ng/mL. Testosterone also only varied over time, increasing from 2.6 ± 0.5 ng/mL at time zero to 7.6 ± 1.5 ng/mL at 60 min after naloxone treatment.

Second Year

No significant differences in LH or testosterone were observed between 0.75 and 1.50 mg of naloxone/kg of BW.

The covariate for LH before naloxone injection was significant ($P < 0.001$). The sexual activity × time interaction observed ($P < 0.02$; Figure 3) seemed to be related to an LH increase ($P < 0.001$) at 15 min after naloxone injection to approximately 1 ng/mL for sexually active rams, whereas LH only increased ($P < 0.01$) to less than 0.5 ng/mL at 15 to 30 min for sexually inactive rams. The LH concentration was maintained at approxi-

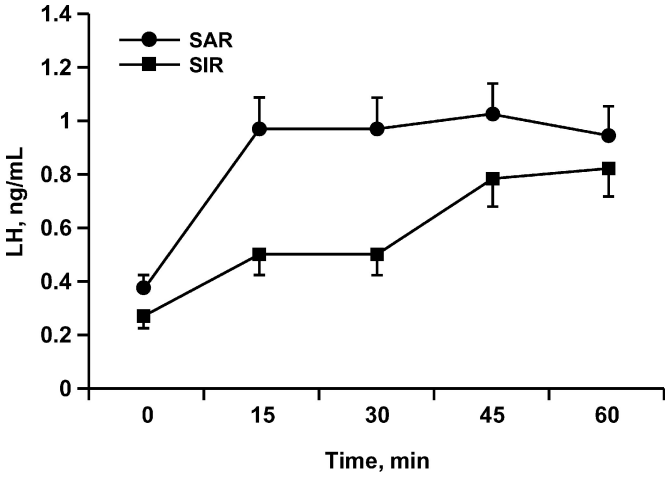

Figure 3. Least squares means for LH after treatment (0.75 or 1.50 mg naloxone/kg of BW) during Year 2 in sexually active (n = 26; SAR [-●-]) and sexually inactive rams (n = 20; SIR [-■-]). A sexual activity × time interaction was observed ($P < 0.02$), with the earlier increase occurring in SAR; only those data are depicted in the figure. The response of LH to the 0.75 or 1.5 mg of naloxone/kg of BW was not different. The estimate of variability is depicted by estimated standard errors of the least squares means because of data transformation.

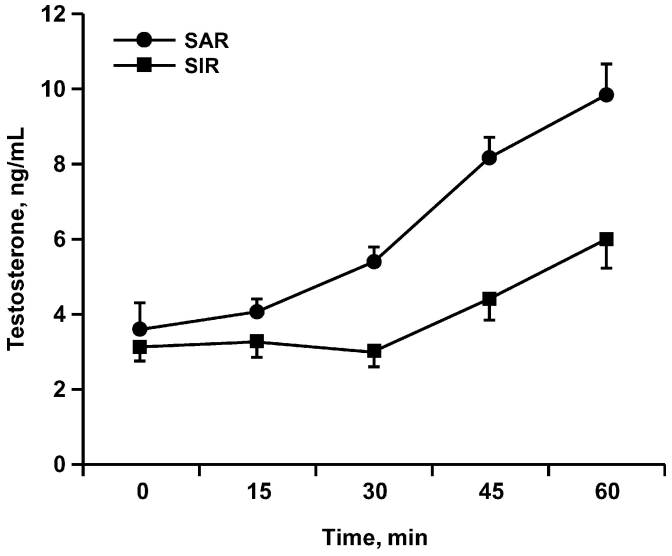

Figure 4. The least squares means for testosterone after treatment (0.75 or 1.50 mg of naloxone/kg of BW) during Year 2 in sexually active (n = 26; SAR [-●-]) and sexually inactive rams (n = 20; SIR [-■-]). A sexual activity × time interaction was observed ($P < 0.01$) with earlier and greater increase occurring in SAR; only those data are depicted in the figure. The response of testosterone to the 0.75 or 1.5 mg of naloxone/kg of BW was not different. The estimate of variability is depicted by estimated standard errors of the least squares means because of data transformation.

mately 1 ng/mL out to 60 min in sexually active rams, and it increased to approximately 0.8 ng/mL at 45 to 60 min after naloxone in sexually inactive rams.

The covariate for testosterone before naloxone injection was significant ($P < 0.001$). After naloxone injection, a sexual activity × time interaction ($P < 0.01$) was observed (Figure 4). The interaction seemed to be related to testosterone concentration beginning to increase ($P < 0.01$) at 30 min in sexually active rams and not until 45 min after naloxone in sexually inactive rams. At 60 min after naloxone treatment, testosterone was greater (9.9 ± 1.0 vs. 6.0 ± 0.8 ng/mL; $P < 0.01$) for sexually active compared with sexually inactive rams, respectively.

Prediction Data

For the Year 1, the percentage of rams predicted correctly with logistic procedures was 73.5%, with 77.8% for sensitivity and 68.2% for specificity (Table 1) using testosterone values at 60 min after naloxone injection. The combination of using the LH value at 15 min multiplied by the testosterone value at 60 min after naloxone treatment provided an overall correct prediction of 69.4%. Even though testosterone values at 45 min after treatment entered the prediction model at the 10% cutoff, unlike testosterone at 60 min, the prediction accuracy achieved with testosterone at 60 min after naloxone was 73.5% compared with 67.4% with the testosterone concentration at 45 min after naloxone. There were 12 rams incorrectly identified for sexual activity, and they were split equally between sexually active and inactive rams.

For Year 2, the logistic procedure correctly predicted 84.8% of the rams with 81.5 % for sensitivity and 89.5% specificity (Table 2) using the combination of LH at 15 min multiplied by testosterone at 60 min after naloxone injection. The correct predictions achieved with testosterone values alone at 45 or 60 min were 74.5 and 78.3%, respectively. For the prediction based on LH multiplied by testosterone, two sexually active and five inactive rams were incorrectly diagnosed. For the 2 yr, 11 rams out of the 46 rams that were present with all response variable data available differed in their predicted sexual performance for a repeatability of 76.1%.

The prediction equation developed from logistic procedures was logit = intercept estimate + explanatory estimate × explanatory variable value for each animal. The predicted probability (p) = exp (logit)/(1 + exp [logit]). For the highest results in Year 1, p = exp (−1.2755 + 0.2333 × TST8)/[1 + exp (−1.2755 + 0.2333 × TST8)]. For the highest prediction results in Year 2, p = exp (−0.8226 + 0.1264 × [LHST5 × TST8])/{1 + [−0.8226 + 0.1264 × (LHST5 × TST8)]}. Note that equations for highest accuracy varied, indicating that new equations may need be calculated with each future test conducted until a database is developed to determine whether a standard equation can be applied.

Discussion

Reproduction in the male is affected by numerous physiological mechanisms (Haynes and Schanbacher, 1983), including an opioid influence on GnRH (Ebling, et al., 1987) and LH (Malven, 1986) secretion. A single injection of naloxone, an opiate antagonist, to mature rams during the breeding season results in an LH increase followed by an increase in testosterone, which were used as explanatory variables in logistic procedures to predict the sexual activity of rams with a range of 69 to 85% accuracy dependent upon year and explanatory variable used. The greatest accuracy in Year 1 (73.5%) was with testosterone at 60 min after naloxone injection, whereas the combination of LH multiplied by testosterone resulted in an accuracy of 69.4%. The greatest prediction accuracy (85%) was achieved in Year 2, with a combined explanatory variable of LH at 15 min multiplied by testosterone at 60 min after naloxone treatment. The repeatability of the test for Years 1 and 2 on the same rams was 76.1%. These prediction results with logistic procedures are similar to the 77% accuracy combining both years as reported in the patent (Perkins et al., 2001) and differ from the lower accuracies obtained in early postpubertal rams during the breeding season (Stellflug, 2003) and just before the fall breeding season (Stellflug, 2002, 2003).

Table 1. Classification information for predicting sexually active and inactive mature rams given naloxone in December of Year 1[a]

Probability level[d]	Correct[b]		Incorrect[c]				
	Sexually active	Sexually inactive	Sexually active	Sexually inactive	Correct	Sensitivity	Specificity
					%[e]		
0.4	21	12	10	6	67.3	77.8	54.5
0.5	21	15	7	6	73.5	77.8	68.2
0.6	16	17	5	11	67.3	59.3	77.3
0.7	12	18	4	15	61.2	44.4	81.8

[a]Rams (n = 49) were treated with naloxone (1.50 mg/kg of BW) in December. Logistic regression procedures were used to model the probability that rams were sexually active using serving capacity scores as the response variable and LH and testosterone values as explanatory variables. Predictability of sexually active rams was numerically greater than that for sexually inactive rams.
[b]Correct observations for each classification at the corresponding probability.
[c]Incorrect observations for each classification at the corresponding probability.
[d]Probability level represents the level of Type I and Type II errors accepted with 0.05 probability assuming sensitivity and specificity are of equal importance.
[e]Percentages are given for overall correct predictions of sexually active rams. Sensitivity and specificity are percentages of sexually active and inactive rams for each classification at the corresponding probability.

The objective evaluation with logistic procedures to model the probability of correctly identifying sexually active rams rather than the subjective approach reported in the patent achieved either a similar or greater accuracy of prediction and provided a better prediction method.

Obtaining the highest accuracy in Year 2 may be related to age and maturity of rams. Although rams were mature (19 to 20 mo of age) at the time of naloxone treatment in Year 1, perhaps the hypothalamic-pituitary-gonadal axis was still not fully developed and was still less responsive to naloxone disinhibition, as was proposed for early postpubertal rams (Stellflug, 2003). In support of this concept, testosterone is reported to gradually increase in rams until 21 mo of age (Haynes and Schanbacher, 1983), which may be the reason for the increase in testosterone we observed between Year 1 and 2 in the current study (however, year and age were confounded). The increased testosterone in Year 2 and the increased accuracy of predictability in Year 2 was probably not related to seasonality because the early November and December naloxone tests were both during the fall breeding season. Seasonality was reported previously to influence naloxone tests before the breeding season (Stellflug, 2003).

The LH and testosterone concentrations are greater in sexually active than in sexually inactive rams after the naloxone challenge in the current study and when exposed to estrual ewes (Perkins et al., 1992a). This is in contrast to the previous reports (Stellflug, 2002, 2003) that sexually inactive rams had greater LH and testosterone response to naloxone than the sexually active rams before the breeding season. Perhaps sexually inactive rams with a greater LH and testosterone

Table 2. Classification information for predicting sexually active and inactive mature rams given naloxone in November of Year 2[a]

Probability level[d]	Correct[b]		Incorrect[c]				
	Sexually active	Sexually inactive	Sexually active	Sexually inactive	Correct	Sensitivity	Specificity
					%[e]		
0.4	22	10	9	5	69.6	81.5	52.6
0.5	22	17	2	5	84.8	81.5	89.5
0.6	18	17	2	9	76.1	66.7	89.5
0.7	14	17	2	13	67.4	51.9	89.5

[a]Rams (n = 46) were treated with naloxone (0.75 or 1.50 mg/kg of BW) in November. Logistic regression procedures were used to model the probability that rams were sexually active using serving capacity scores as the response variable and LH and testosterone values as explanatory variables. Predictability of sexually active rams was numerically less than that for sexually inactive rams.
[b]Correct observations for each classification at the corresponding probability.
[c]Incorrect observations for each classification at the corresponding probability.
[d]Probability level represents the level of Type I and Type II errors accepted with 0.05 probability assuming sensitivity and specificity are of equal importance.
[e]Percentages are given for overall correct predictions of sexually active rams. Sensitivity and specificity are percentages of sexually active and inactive rams for each classification at the corresponding probability.

response before the breeding season than sexually active rams is related to the inactive rams being at a different state in their testicular cycle than intact rams, similar to what Ebling and Lincoln (1985) reported in the pinealectomized or superior ganglionectomized Soay rams. The surgically altered rams were more sensitive to naloxone as indicated by increased LH concentration because they had a different seasonal testicular cycle than the intact rams (Ebling and Lincoln, 1985). Thus results from the current study support the report (Ebling and Lincoln, 1985) that the response to naloxone is enhanced at the peak of the testicular cycle that occurs during the breeding season in intact rams, whereas the reduced LH and testosterone response after naloxone in the sexually inactive rams during the breeding season helped to differentiate between sexually active and inactive rams. With the naloxone challenge test, male-oriented rams responded with a LH and testosterone pattern that was indistinguishable from sexually active female-oriented rams. In contrast, LH did not increase in male-oriented rams exposed to estrual females (Perkins, et al., 1992a; Alexander, et al., 1999). This supports the concept that most of the faculties related to the naloxone response in male-oriented rams are in place similar to female-oriented sexually active rams. Although the reason male-oriented rams do not respond to estrual females in a manner similar to that of the female-oriented rams is unknown, this difference may be related to the chemical (Perkins, et al., 1995; Resko, et al., 1996; Alexander et al., 2001) and structural (Roselli, et al., 2004) brain differences that have been identified in female- and male-oriented rams. However, in some instances, the differences in brain structure were not found between female- and male-oriented rams, but instead, for low-sexually performing rams compared with the others (Alexander, et al., 2001).

It is unlikely that any single drug test is going to identify 100% of the males with breeding performance problems because there are undoubtedly multiple underlying factors for a ram being sexually inactive. Naloxone acts through the hypothalamo-pituitary axis blocking the inhibitory action of endogenous opiates on GnRH (Ebling, et al., 1987) and LH (Malven, 1986). This mechanism of action explains the increase in LH observed in the current study. The close physiological relationship of LH increase followed by testosterone increases within 40 to 60 min (Sanford et al., 1982, 1984) also supports the observation that testosterone concentrations after naloxone help predict sexual activity. We postulate that the naloxone challenge probably only identifies those rams that are sexually inactive because of differences in this physiological mechanism. The results of the current study indicate that a naloxone challenge can help identify sexually active and inactive mature rams during the breeding season. Further research is required to test the validity of the prediction equations obtained from this study.

Implications

During the breeding season, naloxone can assist with identifying sexually active and inactive rams ranging from 1.5 to 3 yr of age, but it cannot distinguish between sexually active female- and male-oriented rams. This test could be used to identify and exclude sexually low-performing rams from the breeding flock. Further refinement of this test is required to optimize frequency of blood sample collection when used as a supplement to breeding soundness examinations. This test could allow producers to purchase rams that are active breeders and possibly increase pregnancies early in the breeding period.

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